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Acute Dermal Toxicity Potential of Guanidine Nitrate in Male and Female Rabbits

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Steven K. Sano, BA, SGT
and
Don W. Korte, Jr., PhD, MAJ, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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Acute Dermal Toxicity Potential of Guanidine Nitrate in Male and Female Rabbits
(Toxicology Series 85)--Sano and Korte

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 28 Jul 88

Edwin S. Beatrice
COL, MC
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(date)

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<p>The acute dermal toxicity of guanidine nitrate was determined in rabbits by topical application to skin sites with a semioclusive covering for 24 hours. No compound-related deaths or clinical signs were observed at a limit dose of 2 g/kg during this study; however, guanidine nitrate did produce dermal irritation under conditions of the study.</p> <p><i>1. ...</i></p>					
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ABSTRACT

The acute dermal toxicity of guanidine nitrate was determined in rabbits by topical application to skin sites with a semioclusive covering for 24 hours. No compound-related deaths or clinical signs were observed at a limit dose of 2 g/kg during this study; however, guanidine nitrate did produce dermal irritation under conditions of the study.

KEY WORDS: Guanidine Nitrate, Acute Dermal Toxicity, Rabbit Nitroguanidine, Munitions

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PREFACE

TITLE REPORT: Acute Dermal Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TL09

GLP STUDY NUMBER: 84022

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: SGT Steven K. Sano, BA

PATHOLOGIST: LTC Lance O. Lollini, DVM VC
Diplomate, American College
of Veterinary Pathologists

REPORT AND DATA MANAGEMENT: A copy of the final report,
study protocol, retired SOPs,
raw data, analytical, stability,
and purity data of the test
compound, tissues, and an
aliquot of the test compound
will be retained in the LAIR
Archives.

TEST SUBSTANCE: Guanidine Nitrate

INCLUSIVE STUDY DATES: 21 June - 31 July 1984

OBJECTIVE: The objective of this study was to determine the
acute dermal toxicity potential of guanidine
nitrate in male and female New Zealand White
rabbits.

ACKNOWLEDGMENTS

The authors wish to thank Gerald F.S. Hiatt, PhD, Yvonne Johnson, BS, Joy Bauserman, MEd, Richard Katona and Roosevelt Cunningham for their assistance in performing the research. MAJ Earl W. Morgan provided administrative and scientific advice as Project Director for the Nitroguanidine Project.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS
INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84022, was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr. 14 July 88
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MAJ, MSC
Study Director

Conrad Wheeler 14 July 88
CONRAD WHEELER, PhD / DATE
DAC
Analytical chemist

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SGT, USA
Principal Investigator



DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
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ATTENTION OF:

SGRD-ULZ-QA (70-1n)

13 July 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84022

1. This is to certify that in relation to GLP Study 84022, the following inspections were made:

16 April 1984	- Protocol Review
31 July 1984	- Necropsy

2. The institute report entitled "Acute Dermal Toxicity Potential of Guanidine Nitrate in Male and Female Rabbits," Toxicology Series 85, was audited on 6 July 1988.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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Acute Dermal Toxicity Potential of Guanidine Nitrate in Rabbits-Sano and Korte

INTRODUCTION

Guanidine nitrate is an intermediate product in the synthesis of nitroguanidine. Nitroguanidine is a primary component of US Army triple-base propellants and is now being produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, reviewed the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

Objective of Study

The objective of this study was to determine the acute dermal toxicity potential of guanidine nitrate in male and female New Zealand White rabbits.

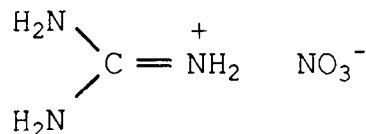
MATERIALS

Test Substance

Chemical name: Guanidine Nitrate

Chemical Abstracts Service Registry No.: 506-93-4

Molecular structure:



Empirical formula: $\text{CH}_5\text{N}_3\text{HNO}_3$

Other test substance information is presented in Appendix A.

Vehicle

The vehicle was sterile saline (0.9% sodium chloride for injection USP, Travenol Laboratories, Inc., Deerfield, IL, Lot No. 7C950X0, Expiration date - October 1985).

Animal Data

Five male and five female young adult New Zealand White rabbits from Elkhorn Rabbitry (Watsonville, CA) were used for this study. The rabbits arrived at LAIR on 21 June 1984 and were identified individually with ear tattoos numbered 84F451-84F455 for males and 84F456-84F460 for females. The animal weights ranged from 2.7 to 3.5 kilograms on receipt and from 2.7 to 3.7 kilograms at dosing. Additional animal data appear in Appendix B.

Husbandry

Rabbits were caged individually in stainless steel, wire mesh cages in racks equipped with automatically flushing dump tanks. No bedding was used in any of the cages. The diet consisted of approximately 150 grams/day Certified Purina Rabbit Chow Diet 5322 (Ralston Purina Company, St. Louis, MO); water was provided *ad libitum* by continuous drip from a central line. The animal room temperature was maintained in a range from 19°C to 22°C with a relative humidity range of 50 to 66 percent with occasional spikes during room cleaning. The photoperiod was 12 hours of light per day.

METHODS

This study was done in accordance with the LAIR Standard Operating Procedure OP-STX-30, "Acute Dermal Toxicity Study" (2), and EPA guidelines HG-Acute-Dermal, "Acute Exposure, Dermal Toxicity" (3).

Group Assignments/Acclimation

The rabbits were quarantined/acclimated for 25 days before the study. During quarantine, they were given one application of Canex mineral oil (Pitman-Moore, Inc., Washington Crossing, NJ) for ear mite prevention. After quarantine, the rabbits were maintained in the same toxicology animal room for the remainder of the study. The hair on the exposure site was clipped 24 hr before dosing. No randomization was necessary as there was only one dose level.

Dosage Levels

A "limit test" was conducted in which 5 male and 5 female rabbits were assigned to the test group that received 2 g/kg of guanidine nitrate applied to the dorsum.

Compound Preparation

According to body weight, 6.19 to 7.49 g of test compound was mixed with 10 ml of isotonic sodium chloride to form a paste. Preparation of the dosing material was performed immediately before application to the animal. Previous testing showed guanidine nitrate to be stable in an aqueous vehicle for a period exceeding the time required to prepare and apply this dosing material (Appendix A).

Test Procedures

The application sites on the dorsal and lateral sections of the animal (roughly 300 cm² in area) were close-clipped with electric clippers (Oster Model A5, Size 40 blade, Sunbeam Corp., Milwaukee, WI) 24 hours before applying the test compound. The compound was placed under a 7-in by 7-in piece of gauze dressing (Curity cover sponges, Kendall Co. Hospital Products, Boston, MA) which was then taped to the back with hypoallergenic tape (Durapore Surgical Tape, 3M Corp., St. Paul, MN). The trunk of the animal was then wrapped with Vetrap[®] bandaging tape (Animal Care Products, 3M Corp., St. Paul, MN) to hold the patch in place and prevent the animal from ingesting the compound. The Vetrap[®] was anchored in place cranially and caudally by strips of Conform[®] elastic tape (Kendall Co. Hospital Products, Boston, MA). The wrappings were left in place for 24 hr. When the wrappings were removed, the exposed area was wiped with a piece of gauze moistened with 0.9% saline to remove any remaining compound.

Observations

Observations for mortality and signs of acute toxicity were performed daily using the following procedure: (1) animals were observed undisturbed in their cages, (2) animals were removed from their cages and given a physical examination, and (3) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Observations were recorded daily for the remainder of the two-week test period. A second "walk through" observation was performed each day with only significant observations recorded. The application site was examined 1/2, 24, 48, and 72 hr after

patch removal, and all lesions were noted and graded. Animals were weighed seven times over the study test period. During evaluation of the exposure site, the intensity of each dermal reaction was graded according to a scale which included five categories to describe severity. Severity was defined as very slight, slight, moderate, well-defined, and severe. At the end of the 14-day period, animals were sacrificed with sodium pentobarbital and evaluated at necropsy. Skin was taken from the exposed area and examined microscopically.

Duration of Study

The study period was 14 days and was preceded by a 25-day quarantine/acclimation period. Appendix C contains a complete listing of study events.

Changes/Deviations from Procedures

An additional eleven days were added to the quarantine period for the animals to become acclimated to environmental conditions. Animals were fed approximately 150 g/day of Certified Purina Rabbit Chow 5322 instead of 110 g/day. These changes did not have a significant effect on the study.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

RESULTS

Clinical Observations

During the course of the study, clinical observations were split into two major categories: those related to the general health of the animal and those related to skin exposure.

Systemic: None of the clinical systemic signs were interpreted as signs of toxicity attributable to the test compound (Table I). Dermal administration of guanidine nitrate had no effect on body weight (Table II).

Dermal: Signs associated with dermal toxicity included erythema, edema, and necrosis (Table I). Dermal irritation was scored at 1/2, 24, 48, and 72 hr for edema and erythema. Results are presented in Table III. The most

TABLE I

Acute Dermal Toxicity of Guanidine Nitrate in Rabbits
Summary of Systemic and Dermal Signs

Animal Number	Dermal Signs	Days of Study	Systemic Signs	Days of Study
MALES				
84F451	Erythema	1		
84F452	Erythema	2		
	Edema	1		
84F453	Erythema	2		
84F454	Edema	2		
	Erythema	5		
	Necrosis	6		
	Scab	10		
84F455	Erythema	2		
FEMALES				
84F456	Erythema	2	Mucus, perianal	1
	Tape Irritation	2	region	
84F457	Erythema	2		
84F458	Erythema	5		
	Tape Irritation	1		
84F459	Erythema	2	Increased startle reflex	1
84F460	Tape Irritation	1	Off feed	1
	Erythema	2		
	Edema	2		

TABLE II
Acute Dermal Toxicity of Guanidine Nitrate in Rabbits
Summary of Body Weights (g)

Animal No.	Day of Study						
	Q0*	Q7	Q14	Q21	0	7	14
MALES							
84F451	3265	3218	3315	3395	3395	3535	3505
84F452	3455	3399	3397	3666	3588	3790	3823
84F453	3526	3477	3551	3792	3717	3769	3872
84F454	3452	3430	3502	3624	3491	3670	3744
84F455	3207	3237	3247	3464	3390	3477	3586
Mean	3381	3352	3402	3588	3516	3648	3706
± S.E.M.	±61	±52	±56	±71	±62	±62	±70
FEMALES							
84F456	2705	2842	2890	3082	3084	3212	3399
84F457	2875	3141	3108	3317	3278	3497	3618
84F458	2949	2965	2975	3142	3190	3281	3423
84F459	2746	2767	2818	2960	2742	2858	2993
84F460	2899	2950	2990	3240	3332	3218	3437
Mean	2835	2933	2956	3148	3125	3213	3374
± S.E.M.	±47	±63	±49	±62	±105	±103	±103

* Q = Quarantine period.

TABLE III

Acute Dermal Toxicity of Guanidine Nitrate in Rabbits

Dermal Irritation Scores
(Hours After Patch Removal)

Animal No.	Erythema				Edema			
	1/2	24	48	72	1/2	24	48	72
MALES								
84F451	1	0	0	0	0	0	0	0
84F452	1	1	0	0	0	1	0	0
84F453	1	1	0	0	0	0	0	0
84F454	2	2	1	1	1	2	0	0
84F455	1	1	0	0	0	0	0	0
FEMALES								
84F456	1	3	0	0	0	0	0	0
84F457	1	1	0	0	0	0	0	0
84F458	1	1	1	1	0	0	0	0
84F459	1	1	0	0	0	0	0	0
84F460	1	2	0	0	1	2	0	0

Erythema Scores:

Value	
None	0
Very slight	1
Slight	2
Moderate	3
Well-defined	4
Severe	5

Edema Scores:

Value	
None	0
Very slight	1
Slight	2
Moderate	3
Well-defined	4
Severe	5

notable sign of dermal irritation was erythema, which was apparent on all animals at the 1/2-hr observation. By 72 hr, the erythema had disappeared in all but two rabbits. Edema occurred on two of the animals at the 1/2-hr observation, three animals at the 24-hr observation, and no animals at the 48-hr observation. Necrosis was the most serious dermal reaction. On one male (84F454), the necrotic area was 2 cm in diameter and persisted as a scab for the entire 14 days of the study.

Gross Pathological Observations

Guanidine nitrate did not produce pathological changes observable at gross necropsy. Sections taken of treated and control skin were examined microscopically, and no lesions were found. A copy of the pathology report is presented in Appendix D.

DISCUSSION

Guanidine nitrate was not lethal to rabbits dosed at 2 g/kg body weight for an acute dermal toxicity test. The acute dermal toxicity test also indicated that this dose of guanidine nitrate did not cause any other systemic toxicity when applied to approximately 10% of the rabbits' body surface area. Dermal irritation was observed at the site of exposure. Guanidine nitrate has been shown to produce severe dermal irritation (4); however, it was administered at considerably higher concentrations than in the present study. Necrosis and eschar formation in one animal could be explained as a localized concentration of the guanidine nitrate under the patch or as an area that was shaved more closely and therefore was more sensitive to the irritative effects of guanidine nitrate. Failure of the microscopic examination to reveal any lesions in the skin of the animal with a scab suggests that the skin under the scab had already healed.

CONCLUSION

Guanidine nitrate was not lethal to rabbits following dermal exposure for 24 hours at a "limit dose" of 2 g/kg. Guanidine nitrate was a dermal irritant under conditions of the study.

REFERENCES

1. Kenyon KF. A data base assessment of environmental fate aspects of nitroguanidine. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1982. DTIC No. ADA125591.
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3. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, dermal toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.
4. Hiatt GFS, Morgan EW, Brown LD, Lewis CM, Johnson YC, Mullen L, Bauserman JW, Okerberg CV, Lollini LO, Korte DW. Acute toxicity of guanidine nitrate and nitroguanidine. 1985 JANNAF Safety and Environmental Protection Subcommittee Meeting. Laurel, Maryland: Chemical Propulsion Information Agency, 1985; CPIA Publication No. 436; pp. 321-330.

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Appendix A: CHEMICAL DATA

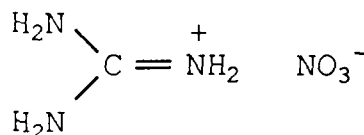
Chemical Name: Guanidine Nitrate

Lot Number: 123820

Chemical Abstracts Service Registry No: 506-93-4

LAIR Code: TP030

Chemical Structure:



Molecular Formula: $\text{CH}_6\text{N}_3\cdot\text{NO}_3$

Molecular Weight: 122.1

Physical State: White crystalline powder

Melting Point: 214°C^1

Analytical Data:

Infrared spectrophotometry was performed, and the spectrum obtained² was identical to the Sadtler spectrum³ for Guanidine Nitrate. Major absorption peaks were observed at 3400 (broad), 3200, 1665, 1575, 1400, 1385, and 825 cm^{-1} . The grade of material obtained for this study is referred to as the Ultralog Grade by the manufacturer. The label on the bulk container states that the purity is at least 99.99%.

Source: Chemical Dynamics Corporation
Hadley Road, P.O. Box 395
South Plainfield, NJ

¹Windholz M, ed., The Merck Index. 9th ed., Rahway, NJ: Merck and Co., Inc., 1976: Monograph Number 4414.

²Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p. 62. Letterman Army Institute of Research, Presidio of San Francisco, CA.

³Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #14498.

Appendix A (cont.): CHEMICAL DATA

Stability:

The stability of guanidine nitrate in aqueous solution is demonstrated by the absorbance values obtained for a standard solution containing 20 µg/ml of guanidine nitrate. This solution was prepared on 25 May and kept at room temperature over the period of analysis. From 25 May to 6 June, four assays of this solution were performed yielding statistically identical absorbance values.⁴ Since the Voges-Proskauer assay is specific for unsubstituted and mono-substituted guanidines, the data demonstrate that aqueous solutions of guanidine nitrate are stable for a period of at least 12 days (Table 1).

TABLE 1: Stability Assay of a 20 µg/ml Standard Solution of Guanidine Nitrate

Date of Analysis	Absorbance Values*
25 May 84	1.74 ± 0.02
29 May 84	1.76 ± 0.05
30 May 84	1.76 ± 0.02
6 Jun 84	1.76 ± 0.02

* Values are mean ± S.D. for three replicates.

⁴ Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 55-57,59. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White

Rationale for Selection: The New Zealand White rabbit is a proven mammalian model for acute dermal studies because of its size, ease of restraint, and skin permeability.

Source: Elkhorn Rabbitry
565 Starr Way
Watsonville, CA 95076

Pretest Conditioning:

- a. Arrival at LAIR 21 June 1984, quarantine time 25 days.
- b. Animals clipped 24 hr before dosing.

Restraint: Manual restraint during application. After dosing the animals were placed in their cages; the bandages were not disturbed over the 24-hr period.

Sex: Male and Female

Age: Young adult

Condition of Animals at Start of Study: Normal

Identification Procedure: Ear Tattoo IAW SOP OP-ARG-1.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
21 Jun 84	Animals arrived at LAIR. They were weighed, tattooed, and examined for illness. Animals held for a two-week quarantine period.
21 Jun 84 - 16 Jul 84	Animals were observed daily and findings were recorded.
28 Jun 84 5,12 Jul 84	Animals were weighed.
16 Jul 84	Animals were close-clipped.
17 Jul 84	Animals were weighed and dosed. Observations and clinical signs were recorded.
17-20 Jul 84	1/2, 24, 48, and 72 hr scoring were performed.
18-31 Jul 84	Animals were observed twice daily for two weeks.
17,24,31 Jul 84	Animals were weighed.
31 Jul 84	Feed was removed during morning observation. Animals were euthanized, necropsied, and a skin sample from application site was preserved for microscopic evaluation.

Appendix D: PATHOLOGY REPORT

PATHOLOGY REPORT

GLP Study 84022

Acute Dermal Toxicity (LD₅₀) test.

Substance: Guanidine Nitrate (CAS #506-93-4) Dose 2 grams/kg.

Species: Rabbit, New Zealand White.

Age: Young adult 5 male, 5 female

History: See LAIR SOP-OP-STX-30 "Acute Dermal Toxicity". All animals were euthanatized.

Gross Necropsy findings:

One male ID# 84F455 had two (2 mm in diameter) white spots in the liver capsule and purulent left otitis media.

Microscopic findings: No lesions were seen in test or control tissues.

Animal ID#	LAIR Accession#	Sex
84F451	35858	M
84F452	35859	M
84F453	35860	M
84F454	35861	M
84F455	35862	M
84F456	35863	F
84F457	35864	F
84F458	35865	F
84F459	35866	F
84F460	35867	F

Comments: No compound related lesions and/or deaths were seen. The otitis and two white spots in the liver of one male were considered incidental findings.



LANCE O. LOLLINI, DVM
LIC, VC
Chief, Pathology Services

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